BENZENESULPHONATE SALT OF A MORPHOLINE UREA DERIVATIVE FOR USE AS A CCR-3 ANTAGONIST IN THE TREATMENT OF INFLAMMATORY CONDITIONS

Novel Compound

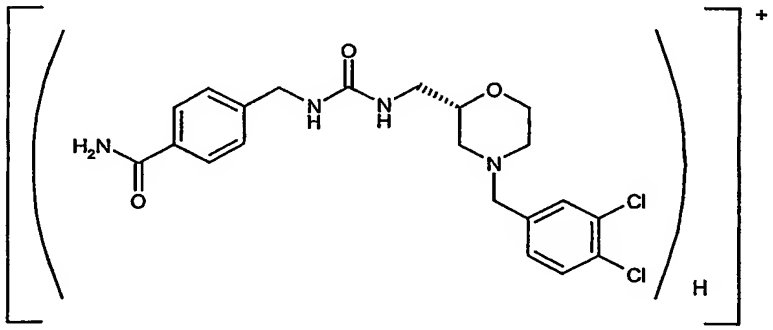
This invention relates to a novel chemical compound, processes for its preparation, pharmaceutical formulations containing it, and its use in therapy.

Co-pending International Patent Application number PCT/GB01/04530 (Glaxo Group Limited) relates to certain morpholine urea derivatives which block the migration/chemotaxis of eosinophils.

It has now surprisingly been found that a specific compound falling within formula (I) of PCT/GB01/04530 has particularly advantageous physicochemical properties, more suitable for the preparation of large scale quantities and for use in the preparation of pharmaceutical formulations. In particular, the compound is crystalline and non-hygroscopic, stable, and displays good solubility profiles.

Specifically, the crystalline nature of the compound is ideal for isolation and purification and is sufficiently stable for use in conventional pharmaceutical formulations. These advantages confer significant benefits of formulation and handling.

Thus, according to one aspect of the invention, there is provided a compound of formula (I):



$A^-.n(ROH)$ (I)

wherein A^- represents the benzenesulphonate (besylate) anion;.

R represents H or C_{1-6}alkyl; and

n is a number from 0.8 to 2.2.

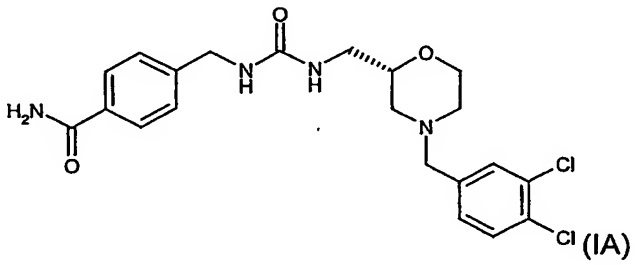
Preferably R represents H.

Preferably n represents a number between 1.1 and 2.1, more preferably about 2.

In a preferred aspect the present invention therefore provides 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}amino)carbonyl]amino} methyl)benzamide benzenesulfonate dihydrate.

The compound 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide is disclosed in co-pending patent application PCT/GB01/04530, however the besylate salt has not previously been disclosed. We have found that the compound 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide does not readily form salts suitable for pharmaceutical use.

In a further aspect of the invention, there is provided a process for the preparation of a compound of formula (I), which process comprises the reaction of a compound of formula (IA);



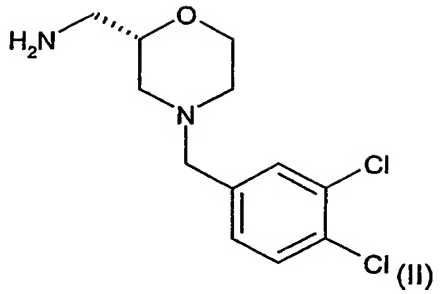
(IA)

with a source of the besylate anion and a suitable C_{1-6}alkanol and water.

Suitable sources of the besylate anion are benzenesulphonic acid and besylate salts such as ammonium besylate. A preferred source of the besylate anion is benzenesulphonic acid.

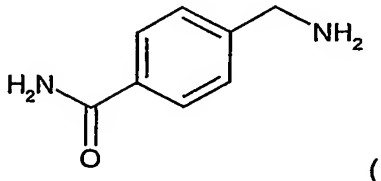
Typically, the compound of formula (IA) is suspended in a suitable C_{1-6} alkanol, suitably ethanol or *iso*-propyl alcohol, and water at elevated temperature, suitably a temperature in the range 35 - 45°C. A solution of the source of besylate anion, preferably benzene sulfonic acid, in water is added. A suitable anti solvent, suitably isopropyl acetate, is optionally added to the solution and the mixture is cooled to 0 - 25°C. A suitable non-polar solvent such as an aliphatic hydrocarbon, e.g cyclohexane may optionally be added. The mixture may optionally be seeded with crystals of the compound of formula (I). The mixture is maintained at a reduced temperature for a suitable period of time to allow crystallisation of the product, and isolated by filtration. Suitable seed crystals of the compound of formula (I) may be prepared by spontaneous crystallisation of a mixture of compound of formula (IA) and benzenesulphonic acid from aqueous C_{1-6}alkanol mixtures at reduced temperature, suitably 0 - 25°C.

The compound of formula (IA) may be prepared by reacting the compound of formula (II), or a salt thereof



(II)

with a compound of formula (III), or a salt thereof



(III)

in the presence of a suitable amine coupling agent, such as N,N'-carbonyldiimidazole.

Typically, a compound of formula (II) in a suitable first solvent is reacted with N,N'-carbonyldiimidazole in the same solvent at reduced temperature, suitably a temperature in the range –10 - 20 °C over a suitable period of time, for example 5 - 60 minutes. Suitable solvents include tetrahydrofuran, dichloromethane, C_{3-4} alkanol, isopropyl acetate, N-methylpyrrolidinone and N,N-dimethylformamide. The mixture is warmed to a suitable temperature, suitably 5 - 30°C and held at this temperature for a suitable period of time, for example 10 - 60 minutes. A suitable solvent, suitably *iso*-propyl alcohol, is added at a suitable temperature, suitably 20-30°C, and held at this temperature for a suitable period of time, for example 15-60 mins. The compound of formula (III) is then added, the mixture heated to a suitable elevated temperature, for example a temperature in the range 40 - 65°C, and stirred for a suitable period of time, for example 60 - 360 minutes. The reaction is then cooled to a suitable temperature, and a suitable second solvent, for example isopropyl acetate, added, followed by a aqueous solution of a suitable acidic salt, such as potassium dihydrogen phosphate, or suitable acid such as acetic acid. The lower aqueous layer removed and the upper organic layer washed with further acid or acidic salt solution, followed by water. The organic phase is distilled at atmospheric pressure to remove the first solvent and leave a slurry or solution of the compound of formula (IA) in the second solvent. This may then be used directly to prepare the compound of formula (I), or filtered to give the compound of formula (IA).

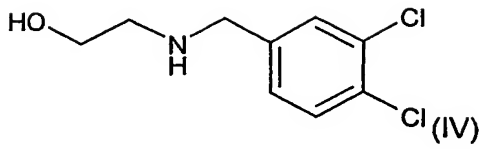
The compound of formula (I) may also be prepared *in situ* by the reaction of a compound of formula (II) or a salt thereof with a compound of formula (III) or a salt thereof, followed by addition of benzene sulphonic acid, suitably an aqueous solution of benzene sulphonic acid. i.e. without isolation of the compound of formula (IA).

Accordingly, there is provided a process for the preparation of a compound of formula (I) which process comprises the reaction of a compound of formula (II) or a salt thereof with a compound of formula (III) or a salt thereof followed by the addition of benzene sulphonic acid, or an aqueous solution thereof, to provide a compound of formula (I).

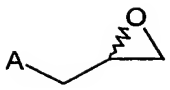
Typically, a compound of formula (II) in a suitable first solvent is reacted with N,N'-carbonyldiimidazole in the same solvent at reduced temperature, suitably a temperature in the range –10 - 20 °C over a suitable period of time, for example 5 - 60 minutes. Suitable solvents include tetrahydrofuran, dichloromethane, C_{3-4} alkanol, *iso*-propyl acetate, N-methylpyrrolidinone and N,N-dimethylformamide. The mixture is warmed to a suitable temperature, suitably 5 - 30°C and held at this temperature for a suitable period of time, for example 10 - 60 minutes. A suitable solvent, suitably *iso*-propyl alcohol, is added at a suitable temperature, suitably 5 – 30°C, and held at this temperature for a suitable period of time, for example, 15-60min. The compound of formula (III) is then added, the mixture heated to a suitable elevated temperature, for example a temperature in the range 40 - 65°C, and stirred for a suitable period of time, for example 60 - 360 minutes. The reaction is then cooled to a suitable temperature, and a suitable second solvent, for example *iso*-propyl acetate, is added, followed by a aqueous solution of a suitable acidic salt, such as potassium dihydrogen phosphate, or suitable acid such as acetic acid. The solution is clarified if necessary, the lower aqueous layer removed and the upper organic layer washed with further acid or acidic salt solution, followed by water. The organic phase is distilled at atmospheric pressure to low volume, a suitable solvent , suitably *iso*-propyl alcohol, is added and the concentration step is repeated. A solution of benzenesulfonic acid in water is added at a suitable temperature, suitably 15-45°C, followed by addition of a suitable anti-solvent, suitably *iso*-propyl acetate. The mixture may be optionally seeded with crystals of the compound of formula (I). Further anti-solvent may be added, the mixture is cooled to 0 – 10°C, and maintained at a reduced temperature for a suitable period. The mixture is then filtered to give the compound of formula (I).

A compound of formula (II) may be prepared by either by Reaction (a), Reaction (b), or Reaction (c).

Reaction (a). Reaction of the compound of formula (IV) with a compound of formula (V)

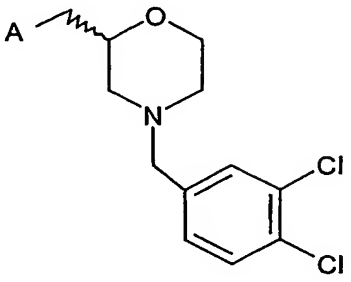


(IV)



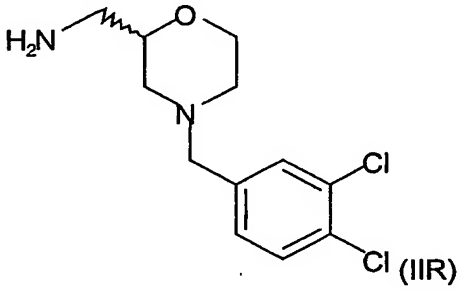
(V)

wherein A is a protected amino group, suitably phthalimido, to give a compound of formula (IIAR)



(IIAR)

wherein A is as previously defined, followed by deprotection of the amino group to give a compound of formula (IIR)

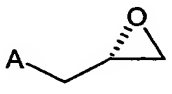


(IIR)

followed by resolution of the resulting enantiomers of the compound of formula (IIR);

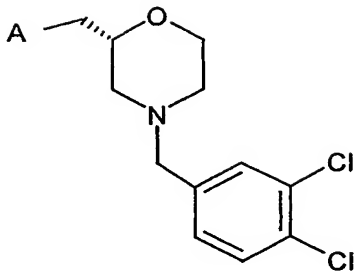
or;

Reaction (b). Reaction of a compound of formula (IV) as hereinbefore defined with a compound of formula (VA)



(VA)

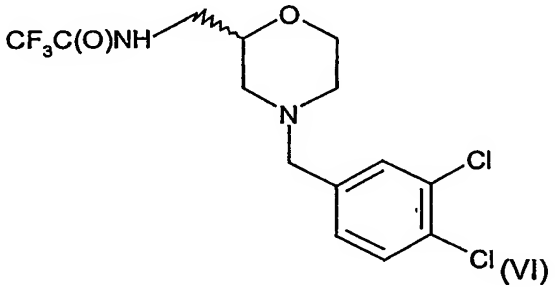
wherein A is as hereinbefore defined for formula (V), to give a compound of formula (IIA)



(IIA)

wherein A is as previously defined, followed by deprotection of the amino group to give the compound of formula (II).

Reaction (c). Hydrolysis of the compound of formula (VI);



(VI)

followed by resolution of the resulting enantiomers of a compound of formula (IIR).

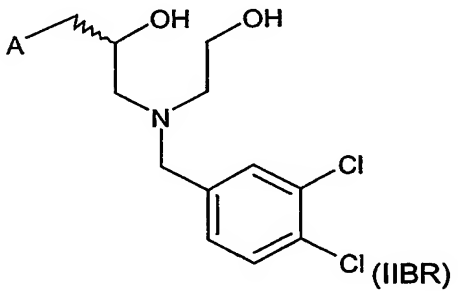
For both reactions (a) and (b), the reaction between the compound of formula (IV) and a compound of formulae (V) or (VA) is typically carried out under the Mitsonobu conditions as follows:

Typically, a mixture of the compound of formula (IV) and the compound of formula (V) or formula (VA) in a suitable solvent, such as tetrahydrofuran or toluene, is stirred, suitably for 2 - 36 hours at a suitable temperature, suitably the reflux temperature of the mixture, under an inert atmosphere, suitably an atmosphere of nitrogen. Further solvent, suitably toluene or tetrahydrofuran, is then added and the mixture cooled, suitably to 0 - 40°C. A phosphine, suitably triphenyl phosphine, is added and the mixture is stirred. An azodicarboxylate, suitably diisopropylazodicarboxylate, is then added over a period of time, suitably 5-120 min, while maintaining the temperature at <40°C. The mixture is allowed to warm, suitably to 20 - 40°C. If necessary, further phosphine and azodicarboxylate reagents can be added. After a further period, the reaction mixture is concentrated to near dryness. A suitable alcohol, suitably propan-2-ol or methanol, is added and the concentration step repeated. This may be repeated as necessary. Further alcohol is then added and the mixture may be heated to a temperature suitably 55 - 75°C. After a suitable period, suitably 20 -

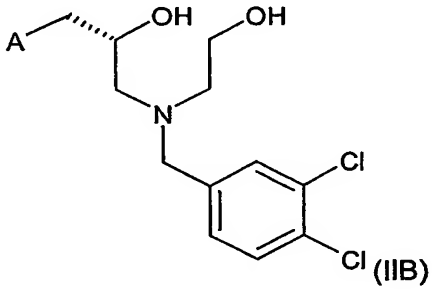
45 minutes, the resultant slurry is cooled, suitably to 15 - 25°C, and then allowed to stand, suitably for 1.5 – 3 hours, after which time the product is isolated by filtration. The filter bed is washed with more alcohol and then dried *in vacuo* at 35 - 45°C to yield the compound of formula (IIAR) or formula (IIA) respectively.

The removal of the protecting group is typically carried out by heating a solution of the compound of formula (IIAR) or formula (IIA) in an appropriate polar solvent, suitably water, in the presence of a mineral acid, suitably concentrated sulfuric acid. The mixture is heated at elevated temperatures, suitably the reflux temperature of the mixture, for a suitable period of time, suitably 8 - 24 hours. The mixture is then cooled, treated with a suitable apolar solvent, suitably dichloromethane, and treated with a base, suitably 0.88G aqueous ammonia, maintaining the temperature below 25°C. The aqueous phase is extracted with further apolar solvent, and the combined organic phase is washed with water. The compound of formula (IIR) or (II) is isolated by evaporation to dryness.

The process for the preparation of the compound of formula (IIAR) or formula (IIA) described above may also be undertaken in two stages, in which an intermediate compound of formula (IIBR) or of formula (IIB) respectively;



(IIBR)



(IIB)

wherein A is as hereinbefore defined for formulae (V) and (VA);
is isolated.

Typically, a mixture of the compound of formula (V) and a compound of formula (V) or formula (VA) in a suitable solvent, such as tetrahydrofuran, C_{3-4} alkanol, toluene, N-methylpyrrolidinone and N,N-dimethylformamide , is stirred, suitably for 2 - 36 hours at a suitable temperature, suitably the reflux temperature of the mixture under an inert atmosphere, suitably an atmosphere of nitrogen. Further compound of formula (V) is added as necessary and the mixture heated at a suitable temperature, suitably the reflux temperature of the mixture, under an inert atmosphere, suitably an atmosphere of nitrogen, for a suitable period of time. The reaction mixture is then cooled, suitably to 20 - 25°C, and the compound precipitated by means of addition of a suitable co-solvent, suitably diisopropyl ether. The compound of formula (IIBR) or formula (IIB)

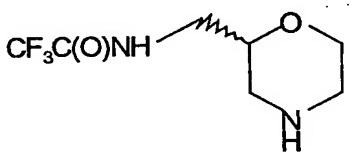
respectively is isolated by filtration, washed with further co-solvent and dried in vacuo.

A compound of formula (IIAR) or formula (IIA) may then be prepared from a compound of formula (IIBR) or formula (IIB) respectively using the conditions described for the reaction between the compound of formula (IV) and the compounds of formulae (V) and (VA) hereinbefore described.

Reaction (c) is typically carried out by stirring a solution of the compound of formula (VI) in a suitable solvent, for example a mixture of methanol and water, and adding a suitable base, for example potassium carbonate. The mixture is stirred at a suitable temperature, for example those in the range 20 - 25°C for a suitable time, for example 16 - 20 hours followed by removal of the organic solvent in vacuo. Water is then added and the mixture extracted with a suitable organic solvent, for example ethyl acetate. The combined organic phases are washed with water and saturated aqueous sodium chloride solution before drying over a suitable drying agent, for example sodium sulphate, filtering and evaporation of the solvent in vacuo. The crude product is then purified by flash chromatography.

The resolution of the compound of formula (II) from the racemic product i.e. the compound of formula (IIR) may be undertaken using techniques well known to those skilled in the art, for example preparative chiral high performance liquid chromatography (chiral HPLC) or by fractional crystallisation of diastereoisomeric salts.

The compound of formula (VI) may be prepared by reaction of the compound of formula (VII)



(VII)

with 3,4-dichlorobenzyl chloride.

The reaction between the compound of formula (VII) and 3,4-dichlorobenzyl chloride is typically carried out in a suitable solvent, for example N,N-dimethylformamide, under an inert atmosphere, for example an atmosphere of nitrogen, with the addition of a suitable base, for example potassium carbonate, and a suitable activating agent, such as sodium iodide. The mixture is then stirred at a suitable temperature, for example a temperature in the range of 20 - 25°C, for a suitable period of time, for example 16 - 20 hours before removing the volatile components in vacuo.

The compound of formula (VII) is prepared by treating a solution of morpholin-2-ylmethylamine in a suitable organic solvent, for example methanol, under an inert atmosphere, for example an atmosphere of nitrogen, with a solution of ethyl-α,α,α-trifluoroacetate in a suitable anhydrous organic solvent, for example diethyl ether. The mixture is then stirred for a suitable period of time, for example 20 - 40 minutes at a suitable temperature, for example a temperature in the range of 20 - 25°C and the volatile components removed in vacuo. The residue is then dissolved in a suitable organic solvent, for example methanol, and the volatile components removed in vacuo.

It is considered that the compounds of formulae (IIA), (IIBR) and (IIB) are new and form further aspects of the present invention.

The compound of formula (IIR) is known (J. Med. Chem., 1991, 34(2), 616-624).

Morpholin-2-ylmethylamine, ethyl-α,α,α-trifluoroacetate, the compounds of formulae (IV) and (V), and the enantiomers of a compound of formula (V) are known, commercially available compounds, or may be prepared by analogy with known procedures, for examples those disclosed in standard reference texts of synthetic methodology such as *J. March, Advanced Organic Chemistry, 3rd Edition (1985), Wiley Interscience.*

Suitable protecting groups in any of the above mentioned reactions are those used conventionally in the art. The methods of formation and removal of such protecting groups are those conventional methods appropriate to the molecule being protected, for example those methods discussed in standard reference texts of synthetic methodology such as *P J Kocienski, Protecting Groups, (1994), Thieme.*

In any of the above described synthetic procedures, Conventional methods of heating and cooling may be employed, for example electric heating mantles and ice/salt baths respectively.

The stability of the compound of the invention may be determined using conventional quantitative analytical methods: For example the stability of the compound in the solid form may be determined by using accelerated stability tests such as differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and isothermal testing at elevated temperatures including conventional storage tests wherein the test compound is stored under controlled conditions of temperature and humidity over known periods of time. Quantitative analysis of the test compounds, against appropriate reference standards before, during and after the storage period allows the stability of the test compound to be determined. For example, the compound of the invention showed no significant degradation in the following tests: 40°C/20% relative humidity, 25 days;

40°C/75% relative humidity, 25 days; 50°C/ambient conditions, 25 days; light cabinet, ambient conditions, 14 days.

As stated the compound of the invention is more soluble in water than the corresponding free base. Thus a convenient method for determining the stability of the compounds of the invention in aqueous solution involves determining the impurity profiles of an aqueous solution of the test compound at known conditions of temperature and over known periods of time. We have found that the compound of formula (I) shows good aqueous stability in presence and absence of light over 8 days at pH range of 2-10.

The quantitative analysis of the impurity profiles of the compound of the invention in the above mentioned tests may be carried out using conventional methods, generally chromatographic methods such as high performance liquid chromatography (HPLC).

The compound of the invention may be tested for in vitro and in vivo biological activity in accordance with the following assays:

(a) CCR-3 Binding Assay

A CCR-3 competition binding SPA (scintillation proximity assay) was used to assess the affinity of novel compounds for CCR-3. Membranes prepared from K562 cells stably expressing CCR-3 (2.5μg/well) were mixed with 0.25mg/well wheat-germ agglutinin SPA beads (Amersham) and incubated in binding buffer (HEPES 50 mM, $CaCl_2$ 1 mM, $MgCl_2$ 5 mM, 0.5% BSA) at 4°C for 1.5 hr. Following incubation, 20 pM of [^{125}I] eotaxin (Amersham) and increasing concentrations of compound (1pM to 30μM) were added and incubated in a 96 well plate for 2 hr at 22°C then counted on a Microbeta plate counter. The total assay volume was 100 μl. Competition binding data were analysed by fitting the data with a four parameter logistic equation. Data are presented as the mean pIC_{50} values (negative logarithm of the concentration of compound which inhibits [^{125}I]eotaxin binding by 50%) from at least two experiments.

(b) Eosinophil chemotaxis Assay.

Compounds were evaluated for their inhibitory effect on eosinophil chemotaxis. Eosinophils were purified from human peripheral blood by standard CD16 cell depletion using a Miltenyi cell separation column and a magnetic Super Macs magnet as previously described (Motegi & Kita, 1998; J.Immunology. 161:4340-6). Cells were re-suspended in RPMI 1640/10% FCS solution and incubated with calcein-AM (Molecular Probes) at 37°C for 30 mins. Following incubation, the eosinophils were centrifuged at 400g for 5 min and re-suspended in RPMI/FCS at 2.2 million/ml. Cells were then incubated in the presence of increasing concentration of compounds (1 pM to 30 μM) at 37°C for

30 mins. For control responses cells were incubated with RPMI/FCS only. The agonist eotaxin (either a concentration response curve or for the functional inhibition curves an EC_{80} concentration) was added to the lower chamber of a 96 well chemotaxis plate (5 μm filter: Receptor Technologies). Eosinophils (50 μl of 2 million/ml cells) were added to the top chamber of the filter plate and incubated at 37°C for 45 mins. Cells remaining on top of the chemotaxis filter were removed and the number of eosinophils which had migrated were quantified by reading the plate on a fluorescent plate reader. Inhibition curves for the effect of compounds on eosinophil chemotaxis were analysed by fitting the data with a four parameter logistic equation. Functional pK_i values (fpK_i) were generated using the equation below (Lazareno & Birdsall, 1995. Br.J.Pharmacol 109: 1110-9).

$$fpKi = \frac{IC_{50}}{1+\left[\frac{[Agonist]}{EC_{50}}\right]}$$

(c) Guinea-pig Ovalbumin Model

Inhibition of Eosinophil Infiltration and Hyper-Reactivity in the Guinea Pig

In a method based on that described by Danahay *et al.*, 1997, ovalbumin sensitised guinea pigs were dosed with mepyramine (30mg kg^{-1} ip) to protect against anaphylactic bronchospasm. Test compounds, dissolved in 10% DMSO and 90% PEG200, were given by the oral route, 30 minutes before ovalbumin challenge (10 minutes breathing of an aerosol generated from a 0.5% solution of ovalbumin). Hyper-reactivity of the airways to the thromboxane mimetic U46619, was measured 24 hours after ovalbumin challenge in un-restrained animals using a whole body plethysmograph (Buxco Ltd., USA). The guinea pigs were then sacrificed and the lungs lavaged. Total and differential leukocyte counts were then obtained for the bronchoalveolar lavage fluid and the percentage reduction in eosinophil accumulation determined (Sanjar *et al.*, 1992). Data were presented as the inhibitory effect of the specified dose expressed as a percentage of the vehicle control response.

Examples of disease states in which the compound of the invention has potentially beneficial anti-inflammatory effects include diseases of the respiratory tract such as bronchitis (including chronic bronchitis), bronchiectasis, asthma (including allergen-induced asthmatic reactions), chronic obstructive pulmonary disease (COPD), cystic fibrosis, sinusitis and rhinitis. Other relevant disease states include diseases of the gastrointestinal tract such as intestinal inflammatory diseases including inflammatory bowel disease (e.g. Crohn's

disease or ulcerative colitis) and intestinal inflammatory diseases secondary to radiation exposure or allergen exposure.

Furthermore, the compound of the invention may be used to treat nephritis, skin diseases such as psoriasis, eczema, allergic dermatitis and hypersensitivity reactions and diseases of the central nervous system which have an inflammatory component (e.g. Alzheimer's disease, meningitis, multiple sclerosis) HIV and AIDS dementia.

Compounds of the present invention may also be of use in the treatment of nasal polyposis, conjunctivitis or pruritis.

Further examples of disease states in which the compound of the invention have potentially beneficial effects include cardiovascular conditions such as atherosclerosis, peripheral vascular disease and idiopathic hypereosinophilic syndrome. Other diseases for which the compound of the present invention may be beneficial are other hypereosinophilic diseases such as Churg-strauss syndrome. Additionally, eosinophilia is commonly found in parasitic diseases, especially helminth infections, and thus the compound of the present invention may be useful in treating inflammation arising from hyper-eosinophilic states of diseases such as hydatid cyst (Echinococcus sp.), tapeworm infections (Taenia sp.), blood flukes (schistosomiasis), and nematode (round worms) infections such as:- Hookworm (Ancylostoma sp.), Ascaris, Strongyloides, Trichinella, and particularly lymphatic filariasis including Onchocerca, Brugia, Wucheria (Elephantiasis).

The compound of the invention may be useful as an immunosuppressive agent and so have use in the treatment of auto-immune diseases such as allograft tissue rejection after transplantation, rheumatoid arthritis and diabetes. Compounds of the invention may also be useful in inhibiting metastasis.

Diseases of principal interest include asthma, COPD and inflammatory diseases of the upper respiratory tract involving seasonal and perennial rhinitis.

Preferred diseases of principal interest include asthma and inflammatory diseases of the upper respiratory tract involving seasonal and perennial rhinitis.

Further diseases also of principle interest include inflammatory diseases of the gastrointestinal tract such as inflammatory bowel disease.

It will be appreciated by those skilled in the art that references herein to treatment or therapy extend to prophylaxis as well as the treatment of established conditions.

As mentioned above, the compound of formula (I) is useful as a therapeutic agent.

There is thus provided as a further aspect of the invention the compound of formula (I) for use as a therapeutic agent, particularly in the treatment of patients with inflammatory conditions, eg. asthma or rhinitis.

According to another aspect of the invention, there is provided the use of the compound of formula (I) for the manufacture of a medicament for the treatment of inflammatory conditions, eg. asthma or rhinitis.

In a further or alternative aspect there is provided a method for the treatment of a human or animal subject suffering from or susceptible to an inflammatory condition eg. asthma or rhinitis, which method comprises administering to said human or animal subject an effective amount of the compound of formula (I).

The compounds according to the invention may be formulated for administration in any convenient way, and the invention therefore also includes within its scope pharmaceutical compositions, comprising the compound of formula (I) optionally with one or more physiologically acceptable diluents or carriers.

There is also provided a process for preparing such a pharmaceutical formulation which comprises mixing the ingredients.

The compound according to the invention may, for example, be formulated for oral, inhaled, intranasal, buccal, parenteral or rectal administration, preferably for oral administration.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch, cellulose or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize- starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycollate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or preservatives, for example, methyl or propyl p- hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compound may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

The compound according to the invention may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or tonicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.

The compound and pharmaceutical compositions according to the invention may also be used in combination with other therapeutic agents, for example antihistaminic agents, anticholinergic agents, anti-inflammatory agents (such as corticosteroids (e.g. fluticasone propionate, beclomethasone dipropionate, mometasone furoate, triamcinolone acetonide or budesonide) or NSAIDs (eg. sodium cromoglycate, nedocromil sodium, PDE-4 inhibitors, leukotriene antagonists, iNOS inhibitors, tryptase and elastase inhibitors, beta-2 integrin antagonists and adenosine 2a agonists)) or beta adrenergic agents (such as salmeterol, salbutamol, formoterol, fenoterol or terbutaline and salts thereof), anti-histamines (eg methapyrilene or loratadine) or antiinfective agents (eg. antibiotics, antivirals).

It will be appreciated that when the compound of the present invention is administered in combination with other therapeutic agents normally administered by the inhaled or intranasal route, that the resultant pharmaceutical composition may be administered by the inhaled or intranasal route.

The compound of the invention may conveniently be administered in amounts of, for example, 0.001 to 500mg/kg body weight, preferably 0.01 to 500mg/kg body weight, more preferably 0.01 to 100mg/kg body weight, 1 to 4 times daily. The precise dose will of course depend on the age and condition of the patient and the particular route of administration chosen.

Biological Data

The compound of formula (I) was tested in the CCR-3 binding and/or eosinophil chemotaxis assays (assays (a) and (b)). The compound of the

invention tested in the CCR-3 binding assay possessed a pIC50 value of greater than 5. The compound of the invention tested in the CCR-3 eosinophil chemotaxis assay possessed an fpKi value of greater than 5.

Throughout the specification and the claims, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer or step or group of integers but not to the exclusion of any other integer or step or group of integers or steps.

The following Examples illustrate the invention but do not limit it in any way.

Description of the Figures

Figure 1 is an X-ray diffraction pattern for a compound of formula (I) dihydrate.

Figure 2 is a combined Thermogravimetric Analysis/Differential Scanning Calorimetry trace for a compound of formula (I) dihydrate;

Figure 3 is a Differential Scanning Calorimetry trace for a compound of formula (I) dihydrate;

Figure 4 is a Thermogravimetric Analysis trace for a compound of formula (I) dihydrate.

General experimental details

NMR

Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker DPX250 or DPX400 instrument.

IR

Infra-red spectra were acquired using a Nicolet Avatar 360 instrument using a Germanium ATR probe

LC/MS System A

The following Liquid Chromatography Mass Spectroscopy (LCMS) system was used: 3mm ABZ+PLUS (3.3cm x 4.6mm internal diameter) column, eluting with solvents: A – 0.1% formic acid + 0.077% w/v ammonium acetate in water; and B – 95:5 acetonitrile:water + 0.05%v/v formic acid, at a flow rate of 3ml per minute. The following gradient profile was used: 100% A for 0.7min; A + B mixtures, gradient profile 0 – 100% B over 3.5min; hold at 100%B for 1.1min; return to 100% A over 0.2min.

LC/MS System B

3μm Phenomenex Luna (50 x 2mm i.d.) column, eluting with solvents: A – 0.05% trifluoroacetic acid in water, B – 0.05% trifluoroacetic acid in acetonitrile, at 40°C and at a flow rate of 1ml per minute. The following linear gradient was used: 0 to 95% B over 8 minutes.

Analytical HPLC column, conditions and eluent

Reverse-phase high performance liquid chromatography was carried out using a Luna 3mm C18(2) (50 x 2.0mm i.d.) column eluting with solvents: A – 100% water, 0.05% TFA; and B – 100% acetonitrile, 0.05%TFA, at a flow rate of 2ml per minute, and at 60°C. The following gradient profile was used:0-95% B over 2.00min, return to 0% B over 0.01min.

Example 1: 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate

4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino} -methyl)benzamide (15g) was suspended in ethanol (60ml) and water (7.5ml) at 40°C. A solution of benzene sulfonic acid (6.0g) in water (7.5ml) was added, followed by addition of further water (15ml). Isopropyl acetate (300ml) was added at 40°C, followed by addition of ethanol (40ml). The mixture was cooled to 0°C, diluted with cyclohexane (10ml) and seeded with authentic 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}amino)carbonyl]amino}methyl) benzamide benzenesulfonate hydrate. The mixture was chilled at 0 °C over 1 h, cyclohexane (100ml) added over 15min and the mixture aged at 0°C. The product was isolated by vacuum filtration, washed with isopropyl acetate (2 x 30ml) and dried in vacuo at 25±5° to give the title compound as a white solid (16.44g).

NMR (DMSO d-6): 2.81δ (1H) broad t; 3.0 – 3.4δ (5H) m; 3.67δ (2H) m; 4.02δ (1H) d of d, J=12.7Hz, 2.5Hz; 4.25δ (1H) d, 5.9Hz; 4.37δ (2H) m; 6.24δ (1H) t, J=5.6Hz; 6,58δ (1H) t, J=5.9Hz; 7.3δ (6H) m; 7.48δ (1H) d of d, J=8.3Hz, 2.0Hz; 7.61δ (2H) m [benzene sulphonate]; 7.75δ (1H) d, J=8.3Hz; 7.81δ (1H) d, 2.0Hz; 7.82δ (2H) m; 7.91δ (1H) broad s; 9.85 (1H) broad s [NH^+].

Example 2: 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate

The slurry of Description 9 was cooled to 50±3° and isopropanol (30ml) added, followed by an aqueous solution of benzene sulfonic acid (32% w/v, 10ml). The mixture was cooled to 22±3° over ca 1h, seeded with authentic 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl) benzamide hydrate and aged at 22±3° for 72 h. The mixture was cooled to 0±3° over 1h and filtered. The filter cake was washed with a 4:1:0.1 mixture of

isopropyl acetate/isopropyl alcohol/water (2.5ml) and dried in vacuo at 25±5° to give the title compound as a white solid (6.9g).

Example 3: 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate

A solution of 1-[(2S)-4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine (60g) in tetrahydrofuran (120ml) was added to a suspension of carbonyl diimidazole (38.8g) in tetrahydrofuran (600ml) over 25min at 0 – 5°C. The mixture was warmed to 10-15°C, and held for 15min. Isopropanol (30ml) was added over 10min, and the mixture was stirred for a further 45min at 10-15°C. 4-Aminomethyl benzamide (35.9g) was added, and the mixture was heated to 55-60°C, and held for 90min. Tetrahydrofuran (240ml) was removed by distillation, and the mixture was cooled to 20-25°C. The mixture was treated with iso-propyl acetate (480ml) and 5% aqueous potassium dihydrogen phosphate (480ml), and the aqueous phase was removed. The organic phase was washed with further 5% aqueous potassium dihydrogen phosphate (2 x 480ml), and finally water (480ml).

The organic phase was concentrated to 250ml by distillation, diluted with isopropanol (850ml), and reconcentrated to a final volume of 420ml. The mixture was cooled to 20-25°C, treated with a solution of benzenesulfonic acid (38.5g) in water (110ml) and warmed to 35°C. Isopropyl acetate (720ml) was added, the mixture was cooled to 20-25°C, and seeded with authentic 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}amino)carbonyl]amino}-methyl) benzamide benzenesulfonate dihydrate. The mixture was stirred for 3h at this temperature, treated with further iso-propyl acetate (180ml), stirred for 30min and cooled to 0-5°C. The product was isolated by vacuum filtration, washed with isopropyl acetate:isopropanol:water (6:1:0.1, 350ml) and dried in vacuo at 35±5° to give the title compound as a white solid (115.6g).

Descriptions

Description 1: 2,2,2-Trifluoro-N-(morpholin-2-ylmethyl)acetamide

To a stirred solution of morpholin-2-ylmethylamine (3.1g) in methanol (70ml) under nitrogen was added an ethereal solution of ethyl-α,α,α-trifluoroacetate (5ml in 20ml ether) which had been washed with saturated aqueous sodium bicarbonate, water and brine, and dried. The mixture was stirred for 30 min at 22°C before removal of all volatiles in vacuo. The residue was dissolved in methanol (10ml) and the volatiles again removed in vacuo to give the title compound as a white crunchy foam (4.9g).

Thermospray Mass Spectrum *m/z* 213 [MH^+].

Description 2: N-{[4-(3,4-Dichlorobenzyl)morpholin-2-yl]methyl}-2,2,2-trifluoroacetamide

To a stirred solution of Description 1 (3.3g) in N,N-dimethylformamide (50ml) under nitrogen was added potassium carbonate (2.46g) and sodium iodide (2.12g). A solution of 3,4-dichlorobenzyl chloride (2ml) in N,N-dimethylformamide (10ml) was added dropwise to the mixture. The mixture was stirred at 22°C for 18h before the volatiles were removed in vacuo. The residue was partitioned between dichloromethane (100ml) and saturated aqueous sodium carbonate solution (50ml). The organic phase was subsequently washed with additional saturated aqueous sodium carbonate solution (2 x 50ml) and water (50ml) before drying over magnesium sulphate, filtering and evaporation of the solvent in vacuo to give a pale yellow oil. The oil was purified by Biotage flash chromatography on a 90g silica cartridge eluting with 25% ethyl acetate in cyclohexane, to give the title compound as a colourless oil (2.97g).
LC/MS (System A) R_t 2.63 min, Mass Spectrum *m/z* 371 [MH^+].

Description 3: [4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine

To a stirred solution of Description 2 (2.97g) in methanol (15ml) and water (5ml) was added potassium carbonate (5.53g). The mixture was stirred at 22°C for 18h before the methanol was removed in vacuo. Water (25ml) was added and the mixture extracted with ethyl acetate (3 x 30ml). The combined organic phases were washed with water (5ml) and saturated aqueous sodium chloride solution (10ml) before drying over sodium sulphate, filtering and evaporation of the solvent in vacuo to give a pale yellow oil. The oil was purified by Biotage flash chromatography on a 90g silica cartridge eluting with 75:8:1 dichloromethane/ethanol/0.880 ammonia solution. The required fractions were combined and the solvent evaporated in vacuo to give the title compound as a colourless oil (1.85g).
LC/MS (System A) R_t 1.77 min, Mass Spectrum *m/z* 275 [MH^+].

Description 4: [4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine (alternative synthesis)

A mixture of 2-[(3,4-dichlorobenzyl)amino]ethanol (0.980g) and 2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione (1.10g) was heated at 80°C under nitrogen for 3h. The resulting solid mass was treated with concentrated sulphuric acid (1.5ml) then stirred at 150°C for 24h. The mixture was treated with water (100ml) then washed with ethyl acetate (2x100ml). The dark aqueous phase was basified to ~pH 12 using 5M aqueous sodium hydroxide, then extracted with ethyl acetate (2x100ml). The combined organic extracts were washed with water and brine,

dried (Na_2SO_4) and concentrated under vacuum to give the title compound as a brown oil (1.02g).

Description 5: 1-[(2S)-4-(3,4-Dichlorobenzyl)morpholin-2-yl]methylamine

Description 3 (racemic mixture, 8g) was separated into its single enantiomers by preparative chiral-HPLC. The separation was carried out using a 2" x 22cm Chiralpak AD 20µm column, Merck self pack DAC system, eluting with 95:5:0.1 (v/v) heptane : absolute ethanol: diethylamine (flow rate: 55ml/min over 40min, UV detection 225nm); sample load preparation: 400mg sample in 20ml 3:2 (v/v) absolute ethanol: system eluent.
The title compound (2.49g) was obtained as follows: preparative HPLC retention time 23.0 min.

Description 6: 1-[(2S)-4-(3,4-Dichlorobenzyl)morpholin-2-yl]methanamine salt with D-tartaric acid 1:1

Description 3 (0.613g) was dissolved in methanol (12.3ml). D-Tartaric acid (0.335g) was added and the slurry was heated to reflux for 50min. The mixture was allowed to cool to 0-5°C and the precipitate isolated by filtration to give the title compound as a white solid (0.4g).
ee: 76%ee
Chiral analytical HPLC (Chiralpak AD column, 4.6 x 250mm, eluent 50:50:0.1 MeOH: EtOH: Butylamine, flow rate 0.5ml/min, UV detection at 220nm), Rt 8.9min.

Description 7: - Preparation of 2-{[(2*R*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}-1*H*-isoindole-1,3(2*H*)-dione

A mixture of 2-[(3,4-dichlorobenzyl)amino]ethanol (2.038 g) and (S)-2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione (2.032g) in tetrahydrofuran (3.3ml) was stirred and heated at reflux under nitrogen. After 21.5h more tetrahydrofuran (12.5ml) was added and the mixture was cooled to 3°. Triphenyl phosphine (2.793g) was added and the mixture was stirred until all the solid had dissolved. Diisopropylazodicarboxylate (2.1ml) was then added over 12min maintaining the temperature at <7°. After 2.25h the mixture was allowed to warm to 22°. After 5.3h more triphenylphosphine (121mg) and diisopropylazodicarboxylate (0.09ml) were added. After 22.5h the reaction mixture was concentrated to near dryness. Propan-2-ol (12ml) was added and the concentration repeated, this was repeated once more. More propan-2-ol (12ml) was added and the mixture was heated to 70°. After 0.5h the slurry was cooled to 22° and then after a further 2h the product was collected. The bed was washed with propan-2-ol (2x4ml) and then dried *in vacuo* at 40° to give the title compound (2.622g).

NMR (DMSO d-6): 1.93δ (1H) d of d, J=11.0Hz, 8.8Hz; 2.10δ (1H) d of t, J=3.5Hz, 11.3Hz; 2.52δ (1H) broad d, J=11.3Hz; 2.77δ (1H) broad d, J=11.3Hz; 3.3 – 3.8δ (7H) m; 7.31δ (1H) d of d, J=8.2Hz, 1.9Hz; 7.55δ (1H) d, J=1.9Hz; 7.68δ (1H) d, J=8.2Hz; 7.86δ (4H) m.

Description 8: Preparation of [(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methylamine

A slurry of Description 7 (1.00g) in water (8.5ml) was heated to 75° and then treated dropwise with concentrated sulphuric acid (2.5ml). The mixture was then heated at reflux. After 23h the reaction mixture was cooled to 22° and then treated with dichloromethane (6ml). 880 Ammonia solution (7ml) was then added dropwise with cooling. More dichloromethane (10ml) was added. The aqueous phase was separated and extracted with more dichloromethane (10ml). The combined organic phase was washed with water (5ml) and then evaporated to dryness. The residue was reevaporated from DCM to give the title compound as an oil (662mg).

NMR (DMSO d-6): 1.78δ (1H) t, J=10.5Hz; 2.06δ (1H) d of t, J=3.4Hz, 11.3Hz; 2.45 – 2.65δ (3H) m; 2.73δ (1H) d of t, J=11.3Hz, 1.7Hz; 3.38δ (1H) m; 3.46δ (2H) AB q; 3.51δ (1H) d of d, J=11.3Hz, 2.5Hz; 3.77δ (1H) d of m, J= 11.3Hz; 7.31δ (1H) d of d, J=8.3Hz, 2.0Hz; 7.55δ (1H) d, J=2.0Hz; 7.58δ (1H) d, J=8.3Hz.

Description 9: 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide

A solution of (5g) in THF (10ml) was added to a slurry of N,N'-carbonyldiimidazole (3.2g) in THF (30ml) at 5-10 °C over ca. 10 min. The mixture was warmed to 15±3° and held at this temperature for ca. 15min. 4-Aminomethyl benzamide (3.0g) was then added, the mixture heated to 60±3° and stirred at this temp for 75 min.

The reaction was cooled to 22±3° and isopropyl acetate (40ml) added, followed by a solution of potassium dihydrogen phosphate (5% w/v, 40ml). The solution was filtered through celite (2g), the lower aqueous layer was removed and the upper organic layer washed with potassium dihydrogen phosphate (5% w/v, 2x40ml) then water (40ml). The organic phase was distilled at atmospheric pressure to remove THF and leave a slurry of 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide in isopropyl acetate (ca 60ml).

The slurry containing 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide may be used directly for the process of Example 2, or filtered to give 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-

yl]methyl} amino)carbonyl]amino}methyl)benzamide in isolated form for use in the process of Example 1.

Description 10: 2-{(2*R*)-3-[(3,4-dichlorobenzyl)(2-hydroxyethyl)amino]-2-hydroxypropyl}-1*H*-isoindole-1,3(2*H*)-dione

To a solution of 2-[3,4-dichlorobenzyl)amino]ethanol (2.8g) in tetrahydrofuran (6.2 ml) is added (S)-2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione (3.1g) with stirring, under a nitrogen atmosphere. The mixture was heated to 90 °C over 1 h, then held at this temperature for 18 h. Further 2-[3,4-dichlorobenzyl)amino]ethanol (0.14g) is added, and the reaction mixture heated to 90 °C for a further 5h. The reaction mixture is cooled to 22 °C, and diisopropyl ether (21ml) added, and the product isolated by vacuum filtration. The filter cake is washed with diisopropyl ether (3 ml) and dried in vacuo at 40 ° to give the title compound as a white solid (4.79g).

LC/MS (System B) R_t 3.85min, Mass Spectrum m/z 423 [MH^+]

Description 11: Preparation of [(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methylamine (alternative deprotection)

A slurry of Description 7 (70.00g) in 40%w/w aqueous methylamine (560ml) was heated to 50-60° and held for 3h. The mixture was treated dropwise with 10N sodium hydroxide (35ml), cooled to <25° and then treated with dichloromethane (210ml). The aqueous phase was separated and extracted with more dichloromethane (210ml). The combined organic phase was washed with water (70ml) and then evaporated to dryness. The residue was reevaporated from DCM to give the title compound as an oil (47.7g).

Description 12: 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide

A mixture of [(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methylamine, 1:1 salt with D-tartaric acid (20g), water (100ml) and dichloromethane (120ml) was treated with aqueous ammonia (10ml) at 15-25°C. The layers were separated, the aqueous layer was washed with dichloromethane (30ml) and the combined organic layers were washed with 2% aqeous sodium chloride (20ml). The organic phase was concentrated to an oil, and taken up in THF (20ml). This solution was added to a slurry of N,N'-carbonyldiimidazole (7.8g) in THF (80ml) at 0-5 °C over ca. 10 min. The mixture was warmed to 15±3° and held at this temperature for ca. 15min. Isopropanol (6ml) was added, and stirred for a further 5min at 15±3°. 4-Aminomethyl benzamide (7.2g) was then added, the mixture heated to 60±3° and stirred at this temp for 120 min.

The reaction was cooled to 22±3° and isopropyl acetate (96ml) added, followed by a solution of potassium dihydrogen phosphate (5% w/v, 96ml). The organic phase was washed with further potassium dihydrogen phosphate (5% w/v, 2 x 96ml), and then water (96ml). The organic phase was filtered through Celite, and the cake was washed with isopropyl acetate. The filtrate was concentrated to a semi-solid and resuspended in isopropyl acetate (200ml) at reflux. The slurry was cooled to 20-25°C over 1 h, cooled to 0-5°C and aged for 15min. The product was isolated by filtration, washed with isopropyl acetate (50ml) and dried in vacuo to give the title compound as a white solid (17.4g).

LC-MS (System A) R_t 2.27min, Mass Spectrum m/z 451/453 (MH+)

Description 13: - Preparation of 2-{[(2*R*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl}-1*H*-isoindole-1,3(2*H*)-dione

A mixture of 2-[(3,4-dichlorobenzyl)amino]ethanol (400g) and (S)-2-(oxiran-2-ylmethyl)-1H-isoindole-1,3(2H)-dione (399.6g) in toluene (1150ml) was stirred and heated at 103-107°C under nitrogen. After 22.5h the mixture was cooled to <60°C and charged with tetrahydrofuran (2800ml). Triphenyl phosphine (548g) was added and the mixture was stirred until all the solid had dissolved, then cooled to 5-9°C. Diisopropylazodicarboxylate (412ml) was then added over 70min maintaining the temperature at <12°. The mixture was warmed to 21-25° and stirred for 1.5h. The reaction mixture was concentrated by distillation to a final volume of 2800ml. Methanol (2800ml) was added and the concentration repeated to a volume of 2800ml. More methanol (2000ml) was added and the mixture was heated to 55°. After 0.75h the slurry was cooled to 18° and then after a further 1h the product was collected. The bed was washed with methanol (2x1200ml) and then dried *in vacuo* at 40° to give the title compound (526.9g).

X-ray diffraction

X-ray diffraction data for (4-({[({[(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate is shown in Figure 1. Table 1 below sets out the instrument and parameters used. Table 2 below sets out the peak listings.

Table 1. Instrument and instrument parameters used for data collection

Manufacturer	Philips Analytical X-Ray B.V. The Netherlands
Diffractometer Type	PW3040
Serial	DY667
Tube Anode	Cu
K-Alpha1 wavelength (Å)	1.54056
K-Alpha2 wavelength (Å)	1.54439
RatioAlpha2:1	0.50000
Divergence Slit	Prog. Div. Slit
Receiving Slit	Prog. Rec. Slit
Monochromator Used	YES
Generator Voltage (kV)	40
Tube Current (mA)	55
File Date & Time	6-Feb-2002 16:41
Data Angle Range (°2Θ)	6.0000 - 45.0000
Scan Step Size (°2Θ)	0.020
Scan Type	CONTINUOUS
Scan Step Time	1.00

Table 2. Peak listings

Angle (°2Θ)	Relative Intensity (%)
2.76106	5.94
5.46331	4.86
8.15276	46.78
10.86567	32.69
12.65525	3.49
13.56421	4.50
13.86964	4.02
14.42106	4.40

Angle (°2Θ)	Relative Intensity (%)
15.16974	3.27
16.30551	20.75
17.00973	3.59
17.25384	3.43
17.86169	3.06
18.60297	11.16
19.03017	100.00
19.31215	8.30
19.78254	9.12
20.81643	7.56
21.78742	17.42
22.21255	13.05
23.77012	8.99
24.12210	9.17
24.51820	98.77
25.60615	26.57
26.02674	18.28
27.29165	15.60
27.84695	11.08
28.40132	12.40
28.77836	8.06
29.06108	9.05
29.34410	11.99
29.70510	7.17
29.92808	9.79
30.32458	6.73
30.85363	19.87
31.34853	9.33
31.92524	12.43
32.87292	29.68
33.64329	9.79
34.51241	10.24
35.94723	12.27
36.60514	7.34
36.92942	10.80
37.79933	7.29
38.57353	28.83

Angle (°2Θ)	Relative Intensity (%)
39.13941	16.12
39.88994	7.27
41.25329	18.40
41.68381	19.20
42.10577	6.85
43.99694	10.82
44.55330	14.98

Differential Scanning Calorimetry

The stability of 4-({[({[(2S)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate was measured using differential scanning calorimetry. Table 3 sets out the instrument and parameters used and the results are shown in Figures 2 and 3.

Table 3

Instrument	Manufacturer / Model:	TA Instruments DSC2920
	Serial No.	M2920-234
Method	Sample pre-treatment	None
	Purge gas identity / flow rate	Nitrogen / 20 ml min^{-1}
	Sample pan type	Pinhole aluminium
	Heating rate	10 °C min^{-1}
	Temperature range	Ambient to 300 °C

Thermogravimetric Analysis

The stability of 4-({[({[(2*S*)-4-(3,4-dichlorobenzyl)morpholin-2-yl]methyl} amino)carbonyl]amino}methyl)benzamide benzenesulfonate dihydrate was measured using thermogravimetric Analysis. Table 4 sets out the instrument and parameters used and the results are shown in Figure 4.

Table 4

Instrument	Manufacturer / Model:	TA Instruments TGA2950
	Serial No.	HA29250-226
Method	Sample pre-treatment	None
	Purge gas identity / flow rate	Nitrogen / 100 ml min^{-1}
	Sample pan type	Open aluminium
	Heating rate	10 °C min^{-1}
	Temperature range	Ambient to 300 °C